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Agriculture Development Fund

FINAL REPORT

GENETIC SYSTEMS FOR IMPROVED COLD TOLERANCE IN ECONOMICS CROP SPECIES

19990360

Funded by
The Agriculture Development Fund
March 2001

Prepared by: University of Saskatchewan

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102-00034/

FINAL REPORT - AGRICULTURE DEVELOPMENT FUND PROJECT 19990360-54BX

GENETIC SYSTEMS FOR IMPROVED COLD TOLERANCE IN ECONOMIC CROP SPECIES

December 31, 2000

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A. ABSTRACT/SUMMARY

This one-year project was started in March 2000 with the long-term objectives of identifying the principle systems that plants use to regulate low-temperature gene response and to isolate key low-temperature induced genes for use in crop improvement. The specific objectives of this project were to: i) Quantify the effect of developmental (vernalization and photoperiod) genes on cold tolerance and related characters. ii) Identify and quantify the major cold tolerance genes. iii) Develop molecular markers for cold tolerance genes. These studies have established that there is a highly integrated developmental regulation of low-temperature tolerance gene response. We have also produced lines that will allow us to identify, map, and characterize the low-temperature tolerance genes in wheat.

B. EXECUTIVE SUMMARY

This project was started in March 2000 with the long term objectives of identifying the principle systems that plants use to regulate low-temperature gene response and to isolate key low-temperature induced genes for use in crop improvement programs that use both classical plant breeding and modern genetic transformation methods. The specific objectives of this project were to: i) Quantify the effect of developmental (vernalization and photoperiod) genes on cold tolerance and related characters. ii) Identify and quantify the major cold tolerance genes. iii) Develop molecular markers for cold tolerance genes. Funding was only awarded for year one of a three-year proposal.

The effect of developmental (vernalization and photoperiod) genes on cold tolerance

Exposure to low temperature produces changes in just about every character that can be measured in a plant. These complicated responses have made it difficult to separate cause-and-effect adjustments to low temperature. The research in this project focused on the complex regulatory mechanisms that plants have evolved for the efficient integration of low-temperature gene response into the daily processes of plant survival, growth, and reproduction.

Photoperiod and vernalization requirements are the major characters that influence plant development. Cereals are normally long day (LD) plants in which day length affects apical morphogenesis, leaf production, and other developmental processes. Short day (SD) conditions extend the length of the vegetative phase by increasing the number of leaves and delaying the reproductive phase. LDs accelerate floral initiation and heading by reducing the number of leaves. Vernalization is the acceleration of flowering by a cold treatment. Exposure to temperature in the vernalization range (0 to10°C) shortens the vegetative phase by decreasing the number of leaves in cereals with a vernalization requirement.

Low-temperature (LT) acclimation in cereals is a cumulative process that is initiated at approximately 10°C. The exposure of hardened plants to warm temperature results in rapid de-acclimation. The process of LT acclimation can then be restarted by exposure to temperature in the LT acclimation range. Winter cereal plants gradually lose their ability to tolerate below-freezing temperatures when they are maintained for long periods of time (i.e. over winter) at temperatures in the optimum range for LT acclimation. Research by this program has established that this over-winter

decline in LT response is due to an inability of cereals to maintain LT tolerance genes in an up-regulated state once vernalization and photoperiod requirements have been satisfied. Based on these observations, it is likely that any factor which influences the length the vegetative growth stage affects the expression of LT tolerance genes in cereals exposed to acclimating temperatures. Consequently, timing of the transition from the vegetative to reproductive phase is of fundamental interest not only in terms of flowering time but also in the regulation of LT gene expression.

Results of this study showed that spring-habit photoperiod sensitive genotypes grown under floral-inductive conditions (LD) reached their reproductive phase very quickly and were unable to develop a high level of LT tolerance. In contrast, there was an increased level and longer retention of LT tolerance due to a delay in the transition from the vegetative to the reproductive phase when photoperiod sensitive cereals without a vernalization requirement (spring-habit genotypes) were grown under non-inductive (SD) flowering conditions. A vernalization requirement in winter wheat lengthened the vegetative phase and allowed the expression of LT tolerance genes for an extended period prior to the onset of severe LT stress. An understanding of this relationship has allowed us to construct a field validated winter survival model that successfully simulates over-winter changes in LT tolerance of a wide range of genotypes.

The vernalization saturation point (transition from the vegetative to the reproductive stage) was unaffected by day-length in the present studies. However, non-inductive flowering conditions (SD) delayed the development in plants exposed to LT by increasing final leaf number. This delay, as indicated by shoot apex development under warm temperatures, was reflected in longer retention of LT tolerance. The greater LT tolerance of SD treated winter wheat plants also indicates that the loss in LT tolerance of plants held at temperatures in the acclimation range is not normally due to day-length dependent photosynthate accumulation, depletion of energy reserves after over wintering, or metabolic disturbances.

Winter cereals have a vernalization requirement conditioned by the *vrn* alleles and acclimate upon exposure to LT. They continue to LT acclimate until vernalization saturation after which there is a loss of LT tolerance. The linkage of LT tolerance expression to phenological development adapts the plant to the environment for which it was selected or in which it evolved. For example, a high level of LT tolerance is no longer required after the onset of warm conditions in the spring when rapid growth and reproduction begin. Consequently, satisfaction of the vernalization requirement results in a decline in LT tolerance of over-wintering cereals. Differences in the stage of phenological development also provide an explanation for the observations that partially vernalized plants collected from the field in the fall have the ability to re-acclimate while over-wintered cereals (presumably fully vernalized) are unable to re-acclimate in the spring. A cultivar (such as Dicktoo barley) with high sensitivity to SD photoperiod, but no vernalization requirement, can also achieve the maximum LT tolerance found in that species. In winter-habit genotypes, photoperiod sensitivity influences LT tolerance gene expression even before vernalization saturation. This implies that the vernalization process is progressive and plant development can be influenced by photoperiod during the vernalization process.

This research program has produced several significant advances that have changed the focus of LT genetic studies from the search for structural genes to the investigation of genetic

systems that regulate the expression of the structural genes. These advances can be summarized as follows. **a)** LT gene expression is dependent on the stage of phenological development. **b)** Regulatory controls associated with vernalization and photoperiod sensitivity genes are the key factors responsible for the duration of expression of LT tolerance genes. **c)** Gene(s) closely associated with the vernalization and photoperiod gene complexes have a regulatory effect on LT induced genes. These studies have established that there is developmental regulation of LT tolerance gene response emphasizing the need for functional genomic studies to focus on the genetic mechanisms that determine both phenological development and LT tolerance.

Doubled haploids, genetic control of LT tolerance, and molecular mapping

We have achieved the milestones listed in the research proposed for year one of this project. We have produced lines will allow us to both quantify the effect of *vrn1* the region on LT tolerance and remove the *vrn1* effect so that LT genes outside of the *vrn1* region can be identified, mapped, and characterized. We have also removed the major affect of genes in the *vrn* region by using winter x winter crosses in which the parents differ in LT tolerance.

In this research project we have utilized our available double haploid technology and the appropriate genetic stocks from our carefully screened genetic collection to develop specially designed populations that will allow us to identify and map the genes responsible for differences in LT tolerance in wheat. These studies will also allow us to isolate and determine the genetic control of the different factors determining LT tolerance. Controlled-freeze tests will be employed to identify differences in LT tolerance among the doubled haploid progeny of these crosses. This data will allow us to determine the genetic control of LT tolerance in these populations. DNA will also be extracted from the extremes of the populations and used for Bulked Segregate Analyses utilizing the AFLP technique. Combining these techniques will allow us to identify molecular markers for regions (other than the *vrn* region) that carry genes associated with LT tolerance. Markers (AFLP bands) that have been identified will then be sequenced and primers developed that can be used a) on mapping populations of both barley and wheat to identify chromosomal regions from which they arise and b) in a Marker-Assisted Breeding program to incorporate these chromosomal regions into breeding material without the need for LT screening. Molecular markers linked to LT tolerance genes will also be used in a co-operative project with Dr. R. Chibbar's program at NRC to screen a bacterial artificial chromosome (BAC) library that will be used to isolate and characterize the LT tolerance genes.

Future plans

As mentioned earlier, ADF funding was only awarded for year one of a three-year proposal. This left us without resources to complete the primary objectives of the proposed research. We have looked elsewhere for the necessary support to finish this work and our efforts appear to have been successful.

In co-operation with Dr R. Chibbar from PBI/NRC in Saskatoon, we have applied for an NRC/NSERC grant to complete the genetic control of LT tolerance and molecular mapping studies. This grant application has successfully gone through the peer review process and has been approved by NSERC.

The linkage of genes to function is important if we are going to make use of genomic information to understand the underlying mechanisms of plant response to stress. The unique

genetic and cytogenetic stocks in wheat that we have developed and characterized for LT response allows for novel approaches to genomic studies of abiotic stress. As such, the plant tissue samples that have been collected from the developmental studies and the genetic stocks produced in this project have formed the basis of a Prairie Genome proposal on the functional genomics of abiotic stress that is under final consideration for major funding by Genome Canada.

C. TECHNICAL REPORT

This project was started in March 2000 with the long term objectives of identifying the principle systems that plants use to regulate low-temperature gene response and to isolate key low-temperature induced genes for use in crop improvement programs that use both classical plant breeding and modern genetic transformation methods. The specific objectives of this project were to: i) Quantify the effect of developmental (vernalization and photoperiod) genes on cold tolerance and related characters. ii) Identify and quantify the major cold tolerance genes. iii) Develop molecular markers for cold tolerance genes. Funding was only awarded for year one of a three-year proposal.

The effect of developmental (vernalization and photoperiod) genes on cold tolerance

The objectives of this part of the program were to investigate the expression of low-temperature (LT) tolerance in relation to plant development both before and after transition from the vegetative to the reproductive phase and to determine the interrelationships among photoperiod and vernalization requirements as they affect phenological development and LT tolerance gene expression. Studies to quantify the role that vernalization and photoperiod genes have in the regulation of level and duration of low-temperature gene expression in wheat have been completed and published.

Exposure to low temperature produces changes in just about every character that can be measured in a plant. These complicated responses have made it difficult to separate cause-and-effect adjustments to low temperature. The research in this project focused on the complex regulatory mechanisms that plants have evolved for the efficient integration of low-temperature gene response into the daily processes of plant survival, growth, and reproduction.

Photoperiod and vernalization requirements are the major characters that influence plant development. Cereals are normally long day (LD) plants in which day length affects apical morphogenesis, leaf production, and other developmental processes. Short day (SD) conditions extend the length of the vegetative phase by increasing the number of leaves and delaying the reproductive phase visualised by 'double ridge' formation. LDs accelerate floral initiation and heading by reducing the number of leaves. Vernalization is the acceleration of flowering by a cold treatment. Exposure to temperature in the vernalization range (0 to10°C) shortens the vegetative phase by decreasing the number of leaves in cereals with a vernalization requirement.

Low-temperature acclimation in cereals is a cumulative process that is initiated at approximately 10°C. The exposure of hardened plants to warm temperature results in rapid de-acclimation. The process of LT acclimation can then be restarted by exposure to temperature in the LT acclimation range. Winter cereal plants gradually lose their ability to tolerate below-freezing temperatures when they are maintained for long periods of time (i.e. over winter) at temperatures in the

optimum range for LT acclimation. Research by this program has established that this over-winter decline in LT response is due to an inability of cereals to maintain LT tolerance genes in an up-regulated state once vernalization and photoperiod requirements have been satisfied. Based on these observations, it is likely that any factor which influences the length the vegetative growth stage affects the expression of LT tolerance genes in cereals exposed to acclimating temperatures. Consequently, timing of the transition from the vegetative to reproductive phase is of fundamental interest not only in terms of flowering time but also in the regulation of LT gene expression.

LT response curves were determined for photoperiod sensitive spring and LT tolerant winter wheat and barley cultivars acclimated at 4°C under 8 h short day (SD) and 20 h long-day (LD) photoperiods from 0 to 112 days. Also, three de-acclimation and re-acclimation cycles were used that bridged the vegetative/reproductive transition point for each LD and SD photoperiod treatment of cold tolerant winter wheat cultivars. Spring-habit photoperiod sensitive genotypes grown under floral-inductive conditions (LD) reached their reproductive phase very quickly and were unable to develop a high level of LT tolerance. In contrast, there was an increased level and longer retention of LT tolerance due to a delay in the transition from the vegetative to the reproductive phase when photoperiod sensitive cereals without a vernalization requirement (spring-habit genotypes) were grown under non-inductive (SD) flowering conditions. A vernalization requirement in winter wheat lengthened the vegetative phase and allowed the expression of LT tolerance genes for an extended period prior to the onset of severe LT stress. An understanding of this relationship has allowed us to construct a field validated winter survival model that successfully simulates over-winter changes in LT tolerance of a wide range of genotypes.

Results of our current experiments support our hypothesis that alleles of both vernalization (*Vrn/vrn*) and photoperiod (*Ppd/ppd*) genes affect the rate of phenological development and thereby the expression of LT tolerance genes. They explain field observations, which suggest that *Vrn* and *Ppd* genes accelerate developmental growth rates and reduce LT tolerance. This relationship also makes all LT tolerance associated characters or genes appear to be associated with developmental genes and explains the pleiotropic effects (growth habit and LT tolerance) attributed to developmental genes like *vrn1*.

The vernalization saturation point was unaffected by day-length in the present studies. However, non-inductive flowering conditions (SD) delayed the development in plants exposed to LT by increasing final leaf number. This delay, as indicated by shoot apex development under warm temperatures, was reflected in longer retention of LT tolerance. The greater LT tolerance of SD treated winter wheat plants also indicated that the loss in LT tolerance of plants held at temperatures in the acclimation range was not due to day-length dependent photosynthate accumulation, depletion of energy reserves after over wintering, or metabolic disturbances.

Winter cereals have a vernalization requirement conditioned by the *vrn* alleles and acclimate upon exposure to LT. They continue to LT acclimate until vernalization saturation after which there is a loss of LT tolerance. The linkage of LT tolerance expression to phenological development adapts the plant to the environment for which it was selected or in which it evolved. For example, a high level of LT tolerance is no longer required after the onset of warm conditions in the spring when rapid growth and reproduction begin. Consequently, satisfaction of the

vernalization requirement results in a decline in LT tolerance of over-wintering cereals. Differences in the stage of phenological development provide an explanation for the observations that partially vernalized plants collected from the field in the fall have the ability to re-acclimate while over-wintered cereals (presumably fully vernalized) are unable to re-acclimate in the spring. A cultivar (such as Dicktoo barley) with high sensitivity to SD photoperiod, but no vernalization requirement, can also achieve the maximum LT tolerance found in that species. In winter-habit genotypes, photoperiod sensitivity influences LT tolerance gene expression even before vernalization saturation. This implies that the vernalization process is progressive and plant development can be influenced by photoperiod during the vernalization process.

Tolerance to LT stress is dependent upon the stage of phenological development and the point of transition to the reproductive stage is pivotal in the expression of LT tolerance genes. However, the actual point of reproductive transition is not always clearly visible in the shoot apex morphology of plants grown at LT. The close association found between time to reproductive transition and loss of LT-tolerance supports our hypothesis that plant response to LT acclimation is a function of the degree and duration of expression of LT-tolerance genes and developmental genes are the key factors responsible for duration of expression of LT regulated genes.

This research program has produced several significant advances that have changed the focus of LT genetic studies from the search for structural genes to the investigation of genetic systems that regulate the expression of the structural genes. These advances can be summarized as follows. **a)** LT gene expression is dependent on the stage of phenological development. **b)** Regulatory controls associated with vernalization and photoperiod sensitivity genes are the key factors responsible for the duration of expression of LT tolerance genes. **c)** Gene(s) closely associated with the vernalization and photoperiod gene complexes have a regulatory effect on LT induced genes. These studies have established that there is developmental regulation of LT tolerance gene response emphasizing the need for functional genomic studies to focus on the genetic mechanisms that determine both phenological development and LT tolerance.

Doubled haploids, genetic control of LT tolerance, and molecular mapping

We have achieved the milestones listed in the research proposed for year one of this project. We have developed reciprocal isogenic lines of our most cold tolerant winter wheat cultivar 'Norstar' and the nonhardy spring wheat cultivar 'Manitou', producing in essence winter (*vrn1/vrn1*) and spring (*Vrn1/Vrn1*) habit lines with the genetic backgrounds of both parents. This gives us a winter form of the tender spring wheat cultivar Manitou (winter Manitou) that will carry the Norstar LT genes in the *vrn1* region and a spring form of the hardy winter wheat cultivar Norstar (spring Norstar) that will carry the Manitou LT genes in the *Vrn1* region. These lines will allow us to both quantify the effect of *vrn1* the region on LT tolerance and remove the *vrn1* effect so that LT genes outside of the *vrn1* region can be identified, mapped, and characterized. We have also removed the major affect of genes in the *vrn* region by using winter x winter crosses in which the parents differ in LT tolerance, e.g., Cappelle Desprez (LT_{50} = -13) x Norstar (LT_{50} = -21).

Doubled haploids have an important role to play in increasing the speed and efficiency of the genetic analyses of complex traits. In this research project we have utilize our available double haploid technology and the appropriate genetic stocks from our carefully screened genetic

collection to develop specially designed populations that will allow us to identify and map the genes responsible for differences in LT tolerance in wheat. These studies will also allow us to isolate and determine the genetic control of the different factors determining LT tolerance. We have completed the year one objective of developing doubled haploid populations from the Cappelle Desprez x Norstar and the winter Manitou x Norstar crosses for LT inheritance and mapping studies. Controlled-freeze tests will be employed to identify differences in LT tolerance among the doubled haploid progeny of these crosses. This data will allow us to determine the genetic control of LT tolerance in these populations. DNA will also be extracted from the extremes of the populations and used for Bulked Segregate Analyses utilizing the AFLP technique. Combining these techniques will allow us to identify molecular markers for regions (other than the *vrn* region) that carry genes associated with LT tolerance. Markers (AFLP bands) that have been identified will then be sequenced and primers developed that can be used a) on mapping populations of both barley and wheat to identify chromosomal regions from which they arise and b) in a Marker-Assisted Breeding program to incorporate these chromosomal regions into breeding material without the need for LT screening. Molecular markers linked to LT tolerance genes will also be used in a co-operative project with Dr. R. Chibbar's program at NRC to screen a bacterial artificial chromosome (BAC) library that will be used to isolate and characterize the LT tolerance genes.

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F. PUBLICATIONS AND PRESENTATIONS

S. Mahfoozi, A. E. Limin, P. M. Hayes, P. Hucl, and D. B. Fowler. 2000. Influence of Photoperiod Response on the Expression of Cold Hardiness in Wheat and Barley. Can. J. Plant Sci. 80:721-724.

S. Mahfoozi, A. E. Limin, and D. B. Fowler. 2001. Influence of Vernalization and Photoperiod Responses on Cold Hardiness in Winter Cereals. Crop Sci. (Accepted).

S. Mahfoozi, A. E. Limin, and D. B. Fowler. 2001. Developmental Regulation of Low-Temperature Tolerance in Winter Wheat. Ann. Bot. (Submitted).

I. ACKNOWLEDGEMENTS

This project gratefully acknowledges the financial support of Saskatchewan Agriculture and Food on official reports.